

AD _____

USAARL REPORT NO. 69-18

EFFECTS OF 2,4 DINITROPHENOL ON PROXIMAL
TUBULAR SODIUM REABSORPTION AND PERMEABILITY TO NONELECTROLYTES
IN THE RAT KIDNEY

By

Stephen W. Weinstein, MAJ, MC

June 1969

U. S. ARMY AEROMEDICAL RESEARCH LABORATORY
Fort Rucker, Alabama



UNCLASSIFIED

ADA691104
Technical Report

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)

US Army Aeromedical Research Laboratory
Fort Rucker, Alabama 36360

2a. REPORT SECURITY CLASSIFICATION

UNCLASSIFIED

2b. GROUP

3. REPORT TITLE

EFFECTS OF 2,4 DINITROPHENOL ON PROXIMAL TUBULAR SODIUM REABSORPTION AND
PERMEABILITY TO NONELECTROLYTES IN THE RAT KIDNEY

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

5. AUTHOR(S) (First name, middle initial, last name)

Stephen W. Weinstein, MAJ, MC

6. REPORT DATE

June 1969

7a. TOTAL NO. OF PAGES

6

7b. NO. OF REFS

11

8a. CONTRACT OR GRANT NO.

b. PROJECT NO. 3AO25601A819

c. Task No. 101 (FY 1969)

d.

9a. ORIGINATOR'S REPORT NUMBER(S)

USAARL REPORT NO. 69-18

9b. OTHER REPORT NO(S) (Any other numbers that may be assigned
this report)

10. DISTRIBUTION STATEMENT

Distribution of this document is unlimited. Qualified requesters may obtain copies from the
Defense Documentation Center (DDC), Cameron Station, Alexandria, Virginia.

11. SUPPLEMENTARY NOTES

12. SPONSORING MILITARY ACTIVITY

US Army Medical Research and Development
Command, Washington, D. C. 20315

13. ABSTRACT

A series of 19 experiments were performed to study the effects of 2,4 dinitrophenol (2,4 DNP), an uncoupler of oxidative phosphorylation, on the capacity of the proximal tubule of the rat kidney to reabsorb isotonic sodium chloride and to limit passive permeation of nonelectrolytes. The technique utilized was sequential photomicrography of split oil droplet microperfusions of surface proximal convolutions. The perfusion fluids were isotonic solutions of saline, mannitol, sucrose and raffinose.

The addition of 2,4 DNP had no effect on isotonic saline absorptive rate. However it increased the rate of reabsorption of the nonelectrolytes.

These results suggest an intimate linkage in the proximal convolution of sodium transport directly to the electron transport system since 2,4 DNP prevents oxidative phosphorylation without inhibiting electron transport. In contrast permeability of this tubular segment to nonelectrolytes is enhanced by 2,4 DNP. At least two mechanistic and two functional explanations are possible for this effect. These are discussed and their implications considered.

DD FORM 1473

REPLACES DD FORM 1473, 1 JAN 64, WHICH IS
OBSOLETE FOR ARMY USE.

UNCLASSIFIED

Security Classification

UNCLASSIFIED

Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Kidney Metabolism Physiology						

UNCLASSIFIED

Security Classification

NOTICE

Qualified requesters may obtain copies from the Defense Documentation Center (DDC) Cameron Station, Alexandria, Virginia. Orders will be expedited if placed through the librarian or other person designated to request documents from DDC (formerly ASTIA).

Change of Address

Organizations receiving reports from the US Army Aeromedical Research Unit on automatic mailing lists should confirm correct address when corresponding about unit reports.

Disposition

Destroy this report when it is no longer needed. Do not return it to the originator.

Distribution Statement

Distribution of this document is unlimited.

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

AD _____

USAARL REPORT NO. 69-18

EFFECTS OF 2,4 DINITROPHENOL ON PROXIMAL
TUBULAR SODIUM REABSORPTION AND PERMEABILITY TO NONELECTROLYTES
IN THE RAT KIDNEY

By

Stephen W. Weinstein, MAJ, MC

June 1969

U. S. ARMY AEROMEDICAL RESEARCH LABORATORY
Fort Rucker, Alabama

U. S. Army Medical Research and Development Command

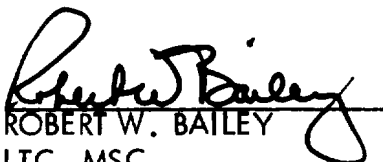
Distribution Statement. Distribution of this document is unlimited.

ABSTRACT

A series of 19 experiments were performed to study the effects of 2,4 dinitrophenol (2,4 DNP), an uncoupler of oxidative phosphorylation, on the capacity of the proximal tubule of the rat kidney to reabsorb isotonic sodium chloride and to limit passive permeation of nonelectrolytes. The technique utilized was sequential photomicrography of split oil droplet microperfusions of surface proximal convolutions. The perfusion fluids were isotonic solutions of saline, mannitol, sucrose and raffinose.

The addition of 2,4 DNP had no effect on isotonic saline reabsorptive rate. However it increased the rate of reabsorption of the nonelectrolytes.

These results suggest an intimate linkage in the proximal convolution of sodium transport directly to the electron transport system since 2,4 DNP prevents oxidative phosphorylation without inhibiting electron transport. In contrast permeability of this tubular segment to nonelectrolytes is enhanced by 2,4 DNP. At least two mechanistic and two functional explanations are possible for this effect. These are discussed and their implications considered.

APPROVED: 
ROBERT W. BAILEY
LTC, MSC
Commanding

EFFECTS OF 2,4 DINITROPHENOL ON PROXIMAL TUBULAR SODIUM REABSORPTION AND PERMEABILITY TO NONELECTROLYTES IN THE RAT KIDNEY

INTRODUCTION:

Previous work from this laboratory provides evidence that oxidative energy metabolism is essential for proximal tubular sodium transport in the nephron of the rat^{(1) (2)}. As a means of exploring the nature of this linkage and to provide evidence as to whether this is based upon the availability of energy from different metabolic sequences or upon the specificity of the form of this energy, the effects of 2,4 DNP on aspects of proximal tubular function were studied. This compound uncouples the process of oxidative phosphorylation from that of electron transport in the mitochondria⁽³⁾. Therefore electron transport with its resultant consumption of oxygen proceeds without the formation of the high energy compounds associated with oxidative phosphorylation.

METHODS:

White Charles River rats weighing 170-310 gms were anesthetized with Inactin 80 mg per Kgm body weight. Jugular vein and tracheal polyethylene cannulae were inserted. The left kidney was prepared for micropuncture as previously described⁽²⁾.

The sequential photomicrographic split droplet microperfusions were performed utilizing the techniques described by Gertz⁽⁴⁾ modified as previously reported from this laboratory⁽²⁾. These techniques are extensively described by Windhager in a recent Monograph⁽⁵⁾.

The photographic recordings were on High Speed Ektachrome, Type B. The resultant slides were utilized to measure the sequential reabsorption of the perfusion fluid droplet. The data was plotted on semilog graph paper as a function of time. The reabsorptive half time ($T_{1/2}$) was obtained for each perfusion sequence.

RESULTS:

In this series of 19 experiments four different perfusion fluids were tested. All except one were studied with and without 2,4 DNP. Table I includes the reabsorptive mean $T_{1/2}$ for each of these. The mean half time for isotonic saline

and for isotonic saline plus 10^{-4} M 2,4 DNP were not statistically different. Their values were 9.2 ± 0.4 seconds and 9.0 ± 0.3 seconds respectively. The control perfusions with mannitol, sucrose, and raffinose, each at a concentration of 300 mM/L showed a progressive increase in $T_{1/2}$ correlating with molecular size. The differences as given in the table are statistically significant.

Addition of 10^{-3} M 2,4 DNP reduced the $T_{1/2}$ means to 13.6 ± 0.7 seconds for mannitol and 18.8 ± 1.1 seconds for raffinose. These were both lower than their respective controls with $P < 0.001$.

The higher concentration of dinitrophenol used with the nonelectrolyte perfusions as compared to that with isotonic saline was required by the observation that initial swelling of the perfused droplet occurs⁽⁴⁾. Since as much as a three-fold dilution occurs as a result of this fluid influx into the tubular lumen, the concentration of 2,4 DNP acting in these nonelectrolyte perfusions is in the range of 3×10^{-4} M to 5×10^{-4} M.

DISCUSSION:

Previous results reported from this laboratory⁽²⁾ suggest that sodium transport in the proximal tubule of the rat is dependent upon oxidative metabolism and independent of glycolysis. The results in the present report support and extend these conclusions.

In the presence of an uncoupler of oxidative phosphorylation such as 2,4 DNP the transfer of energy from the electron transport system through oxidative phosphorylation culminating in the formation of ATP is prevented⁽³⁾. This appears to be accomplished via a shunting of this energy directly from the electron transport system.

On this basis the observation that 2,4 DNP produces no measurable change in proximal tubular isotonic saline reabsorption suggests that the mitochondrial electron transport system contributes energy to proximal tubular sodium transport without a requirement for the products of oxidative phosphorylation. The observations of Urbaitis and Kessler⁽⁶⁾ that 2,4 DNP reduces ATP content and turnover in the mammalian kidney supports this contention.

Since these results differ from those of Chertok et al⁽⁷⁾, who reported inhibition of sodium transport in the proximal tubule of the rat with dinitrophenol, the nonelectrolyte perfusion fluids were tested. The rationale was to provide a maximum sodium gradient and thus observe inhibition of sodium transport that might

otherwise go undetected. In contrast, the results demonstrated a 2,4 DNP enhancement of perfusion fluid reabsorption for mannitol and raffinose solutions.

Two alternatives seem available to explain this result. Since these solutions probably achieve a concentration equilibrium with ions permeable from extracellular fluid(8) (9), reabsorption of the resultant fluid is dependent upon two major factors. The first is the capacity of the sodium pump and the second the permeability of the proximal tubular wall to the particular nonelectrolyte. Enhanced reabsorption could thereby have resulted from increased proximal tubular permeability to mannitol and raffinose or from increased active sodium translocation. Of these two, the evidence supports the permeability change, since enhancement of sodium transport would most likely have shortened the $T_{1/2}$ for isotonic saline reabsorption in the presence of dinitrophenol.

Assuming this reasoning is correct, some functional implications of this data must be considered. If the barriers to passive permeability of sodium ion and of the nonelectrolytes studied are the same in the rat's proximal convolution, passive permeability would be non-rate limiting for sodium reabsorption in this tubular segment. However the distinct possibility remains that the nonelectrolytes and sodium permeate via entirely different routes. In this case a change in permeability to one type of solute could occur without a concomitant modification in permeability to the other.

The mechanism of this effect of 2,4 DNP must also be considered. The two simplest explanations would appear to be first an indirect action on membrane permeability secondary to the $ATPase$ activity of dinitrophenol and second a direct action of this compound on the membrane possibly in a fashion described for artificial membranes(10).

No choice between these two can be made on the basis of presently available evidence. If one speculates, however, that proximal tubular cell permeability is dependent upon oxidative phosphorylation an interesting feedback mechanism evolves. An increase in active sodium transport would decrease cell ATP formation via preferential utilization of energy available from the electron transport system. This would in turn enhance permeability to exogenous substrates, facilitating their entrance into the cell at a time most consistent with metabolic requirements.

The control nonelectrolyte perfusion data are of some interest as differences in permeability appear to correlate with molecular size. However, as indicated by Diamond and Wright(11), nonelectrolyte permeability is dependent upon many

physicochemical factors, making such interpretations difficult. Therefore, more extensive studies would be necessary to provide significant evidence concerning the basis for normal proximal tubular permeability to nonelectrolytes.

In summary, the data provide evidence that the energy for proximal tubular sodium transport in the rat kidney derives directly from the electron transport system rather than the oxidative phosphorylation sequence. The changes in nonelectrolyte perfusion fluid reabsorptive rate with dinitrophenol provide a means to speculate on the metabolic basis for maintenance of proximal tubular cell permeability characteristics. However, insufficient evidence is available to draw specific conclusions regarding the functional or mechanistic basis for this effect.

TABLE I

Perfusion Fluid	T 1/2 + SEM () Seconds	"t" Test
Mannitol 300mM	20.0 ± 1.2 (30)	
Sucrose 300mM	26.6 ± 1.5 (32)	vs. Mannitol .01 P .02
Raffinose 300mM	37.6 ± 1.1 (20)	vs. Sucrose P 0.001
Mannitol 300mM + 10 ⁻³ M 2,4 DNP	13.6 ± 0.7 (30)	vs. Mannitol P 0.001
Raffinose 300mM + 10 ⁻³ M 2,4 DNP	18.8 ± 1.1 (34)	vs. Raffinose P 0.001
Isotonic NaCl	9.2 ± 0.4 (31)	
Isot. NaCl + 10 ⁻⁴ M 2, 4 DNP	9.0 ± 0.3 (33)	vs. Isot. NaCl 0.60 < p < 0.70

5

LEGEND

Effects of 2,4 dinitrophenol on the reabsorption of nonelectrolytes in the proximal tubule

REFERENCES:

1. Weinstein, S. W. and R. M. Klose. Micropuncture studies on energy metabolism and sodium transport in the mammalian nephron. *Am. J. Physiol.* 217: 1969.
2. Weinstein, S. W. USAARL Report No. 69-17, June 1969. Effects of Cyanide and 2 Deoxyglucose on proximal tubular function in the rat kidney.
3. Slater, E. C. in *Comprehensive Biochemistry*. Volume 14. Editors. M. Florkin and E. Stotz. New York, American Elsevier, 1966, Chapter VII.
4. Gertz, K. H., Transtubuläre Natriumchlorid flüsse und Permeabilität für Nichteletrolyte im proximalen und distalen Konvolut der Rattennier. *Arch. ges Physiol.* 276:336, 1963.
5. Windhager, E. E. *Micropuncture Techniques and Nephron Function*. London, Butterworths, 1968, Chapter 3.
6. Urbaitis, B. and R. H. Kessler. Personal communication.
7. Chertock, R. J., W. H. Hulet, and B. Epstein. Effects of cyanide, amytal and DNP on renal sodium absorption. *Am. J. Physiol.* 211:1379-1382, 1966.
8. Kashgarian, M., H. Stöckle, C. Gottschalk, and K. Ullrich. Transtubular electrochemical potential of Na and Cl in the proximal and distal renal tubules of rats during antidiuresis and H₂O diuresis (Diabetes insipidus). *Arch. ges Physiol.* 277:89, 1963.
9. Windhager, E. E. and G. Giebisch. Micropuncture study of renal tubular transfer of NaCl in the rat. *Am. J. Physiol.* 200:581, 1961.
10. Bielawski, J., T. E. Thompson and A. L. Lehninger. The effect of 2,4 dinitrophenol on the electrochemical resistance of phospholipid bilayer membranes. *Biochem. Biophys. Res. Commun.* 24:948-54, 1966.
11. Diamond, J. M. and E. M. Wright. Biological Membranes: The physical basis of ion and nonelectrolyte selectivity. *Ann. Rev. Physiol.* 31: 581-646, 1969.